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RESEALED ERYTHROCYTES: A NOVEL APPROACH TO TREAT CHRONIC DISEASES

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Abstract:

In this area of pharmaceutical innovation, there are more than 30 different drug delivery systems. As people become more interested in consuming safe medications that can be delivered to the intended target spot with few adverse effects, the current environment is concentrating more on targeted drug delivery systems. In actuality, the bio-distribution of medications throughout the body is essentially the fundamental issue with systemic drug delivery. An innovative drug administration method using resealed erythrocytes is now required to solve this issue and increase patient compliance and efficiency. Blood is drawn from the target organism, and erythrocytes and plasma are separated to create carrier erythrocytes. The cells are broken up in a few different ways, the erythrocytes are then hooked up to the drug, they are then resealed, and the resulting carriers are referred to as "resealed erythrocytes". The negative effects of numerous medications, including aspirin, steroids, and cancer treatments, are reduced by resealing erythrocytes. Because they can circulate throughout the entire body and are biocompatible, sealed erythrocytes are becoming more and more important. They also exhibit zero order release kinetics, complete biodegradability, lack of hazardous product, predictable life span, and lowering drug side effects. And these play a big role in treating chronic illnesses like drug addiction, cancer, diabetes mellitus, rheumatoid arthritis, and HIV infection.

Keywords: *Resealed Erythrocytes, Erythrocytes, Erythrosomes, Red Blood Cells, Drug Carriers.*

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INTRODUCTION:

The initial description of erythrocytes as particles "25,000 times smaller than a fine grain of sand"1 was made in the seventeenth century after they were mistaken for fat globules by the Dutch microscopist and Leeuwenhoek. About a century later, Howson discovered these cells to be flat discs rather than globules, providing a more accurate description of them. Hoppe Seyler completed the finding of hemoglobin made by Hünefeld in the 19th century by recognizing its critical function in the supply of oxygen to various tissues. Prior to the late 20th century, reversible oxygenation—along with CO2 exchange—was thought to be the red cell's major or even exclusive activity. The exchange of O2, CO2, hydrogen sulphide, and nitric oxide, immunological clearance, and possibly the clearance of additional soluble blood components like cytokines have all been added to our understanding of erythrocyte function recently.

In 1953, Gardos made the first attempts to entrap chemicals in erythrocytes by trying to load the "erythrocyte ghosts" with ATP. Dextran with molecular weights ranging from 10 to 250 KD was found trapped inside erythrocyte ghosts in 1959, according to Marsden and Ostling. The phrase "carrier erythrocytes" was originally coined in 1979 to refer to the drug-loaded erythrocytes. Fourteen years later, Ihler and Zimmerman independently published the first reports on loading the erythrocyte ghosts with therapeutic substances for delivery purposes. These erythrocytes can be made into drug-loaded carriers by simply taking blood samples from the target organism, isolating the erythrocytes from the plasma, encasing the drug inside the erythrocytes, and then resealing the cellular carriers. These carriers are hence known as resealed erythrocytes. The reaction of these cells under osmotic conditions serves as the foundation for the entire process.

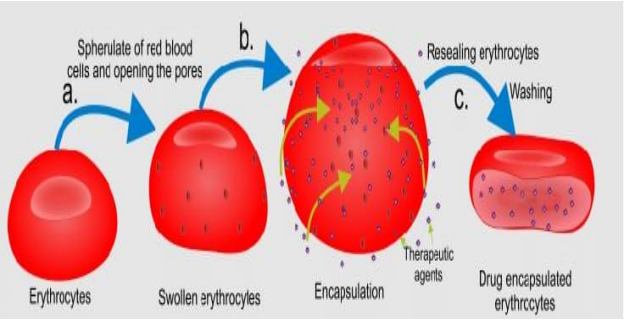
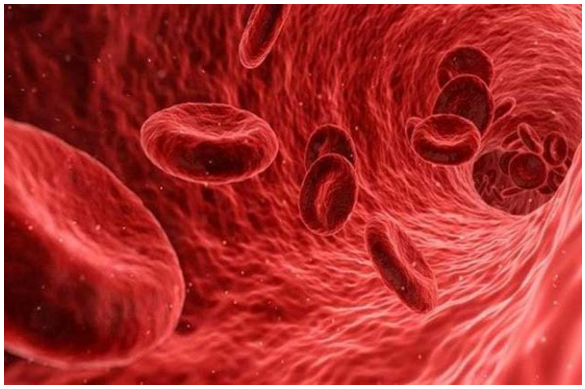


Figure 1: Process of Encapsulated Erythrocytes

MORPHOLPOGY AND PHYSIOLOGY OF ERYTHROCYTES

The most numerous cells in the human body are erythrocytes, which have 5.4 million cells per millimeter in a healthy male and 4.8 million cells per millimeter in a healthy female. Biconcave discs, erythrocytes have a volume of 85-91 m3, an average diameter of 7.8m, a thickness of 2.5m at the perimeter, and a thickness of 1m in the middle. Erythrocytes can



fit through capillaries that are as sample as 3m wide thanks to their flexible, biconcave form.

Figure 2: Erythrocytes

ADVANTAGES OF RESEALED ERTHROCYTES AS DRUG CARRIERS

The following benefits should be present in the resealed erythrocytes:

- Their biodegradability and lack of hazardous product production.
- The carrier's size and shape are remarkably uniform. Relatively unchanging intracellular setting.
- The range of compounds that can be entrapped;
- Protection against endogenous molecules from inactivating the loaded medicine and causing it to degrade.
- The alteration of the drug's pharmacokinetic and pharmacodynamic properties.
- The reduction of concentration fluctuations results from reaching steady-state plasma concentration.
- protection of the body from the harmful effects of medications (e.g. antineoplastic)
- They have the capacity to circulate throughout the body, and there are facilities for handling, transfusing, and working with erythrocytes.
- Avoiding any unwanted immunological reaction against the loaded medication.
- Their capacity to specifically target RES organs.
- optimal zero-order drug release kinetics might be possible.

- The absence of an unfavorable immunological reaction against the medicine that is encapsulated.
- The high amount of medication that can fit inside a little volume of cells guarantees dose sufficiency.
- Optimal conditions and a longer life duration in circulation compared to other synthetic carriers may lead to a life span that is comparable to that of natural erythrocytes.
- Simple control over a life span of a few seconds to several months.
- A reduction in medication side effects.

DISADVANTAGES OF RESEALED ERYTHROCYTES AS DRUG CARRIERS

- Their capacity as carriers to non-phagocyte target tissue is constrained.
- There may be a chance for dosage dumping and cell clumping.

ERYTHROCYTES

ERYTHROCYTES CAN BE USED AS CARRIER IN TWO WAYS

1.focusing on a specific organ or tissue

Only the erythrocyte membrane is employed for targeting. This is accomplished by dividing the cell in a hypotonic solution, adding the medication, and letting the split cells reseal into spheres. Red cell ghosts are the name given to such erythrocytes.

2. For continuous or prolonged release of drugs

Erythrocytes can also be employed as a continuous or prolonged release system, which prolongs the effects of the medicine. There are various ways to encapsulate medications into erythrocytes. They circulate over extended periods of time (up to 120 days) and release the medicine that is entrapped gradually.

SOURCES OF ERYTHROCYTES

Various types of mammalian erythrocytes have been used for drug delivery, including erythrocytes of mice, cattle, pigs, sheep, goats, monkeys, chicken, rats, and rabbits.

ISOLATION OF ERYTHROCYTES

- Vein puncture is used to draw blood into heparinized tubes.
- In a syringe with a drop of anti-coagulant, blood is extracted from a heart or sphenic puncture (in small animals) or through veins (in large animals).
- In a chilled centrifuge, the whole blood is centrifuged at 2500 rpm for 5 minutes at 4°C.
- Carefully removing the serum and Buffy coatings, packed cells are then rinsed three times in phosphate buffered saline (pH=7.4).
- Before usage, the washed erythrocytes are diluted with PBS and kept at 4^0c for up to 48 hours.
- Mammalian erythrocytes of several species, including those from mice, cattle, pigs, dogs, sheep, goats, primates, fowl, rats, and rabbits, have been employed for drug delivery.

Various condition and centrifugal force used for isolation of erythrocytes

Requirement for encapsulation

- In salts, non-polar molecules may become stuck in erythrocytes.
- In general, molecules should be both polar and nonpolar when they are trapped.
- By absorbing over other molecules, hydrophobic compounds can become trapped inside erythrocytes.
- Charged molecules are kept longer after being enclosed than uncharged molecules. When the molecule is smaller than sucrose and larger than β- galactosidase, the size of the molecule that gets captured is a significant determination.

Species	Washing Buffer	Centrifugal Force (g)
Mouse	10 mmol KH_2PO_4 /Na_2HPO_2, pH 7.0 ; 5mmol adenosine ; 5mmol $MgCl_2$; 10 mmol glucose	100-500
Human	154 mmol NaCl or 10mmol KH_2PO_4 / Na_2HPO_4,pH 7.0 ; 2mmol $MgCl_2$; 10 mmol glucose	<500
Rabbit	10 mmol KH_2PO_4 / Na_2HPO_4 pH 7.0	500-1000
Dog	15 mmol KH_2PO_4 /Na_2HPO_4, pH 7.0 ; 5 mmol $MgCl_2$; 10 mmol glucose	500-1000
Cow	10-15 mmol KH_2PO_4/ Na_2HPO_4 ,pH 7.0 ; 2 mmol $MgCl_2$; 10 mmol glucose	1000
Goat	10 mmol KH_2PO_4 / Na_2HPO_4, pH 7.0	500-1000
Horse	10 mmol KH_2PO_4 / Na_2HPO_4, pH 7.0 ; 2mmol $MgCl_2$; 10 mmol glucose	1000
Pig	10 mmol KH_2PO_4 / Na_2HPO_4,pH 7.0	500-1000
Sheep	10 mmol KH_2PO_4 / Na_2HPO_4, pH 7.0 ; 5 mmol $MgCl_2$	500-1000

Table 1: Various conditions and centrifugal forces used for the isolation of erythrocytes

METHODS OF DRUG LOADING IN ERTHROCYTES

Several techniques, such as the electrical pulse approach, osmosis-based systems, and chemical techniques (such as chemical disruption of the erythrocyte membrane), can be used to load medications or other bioactive substances into erythrocytes. Regardless of the technique employed, the ideal characteristics for the compound's successful entrapment include a high degree of water solubility, resistance to erythrocyte degradation, a lack of physical or chemical interaction with the membrane, and clearly defined pharmacokinetic and pharmacodynamic properties.

Types

A] Osmosis based methods

1. Hypotonic Hemolysis
2. Hypotonic Dilution
3. Hypotonic Dialysis
4. Hypotonic Pre-Swelling

B] Chemical perturbation of the membrane

C] Electro-insertion or electro encapsulation

D] Entrapment by endocytosis

A] OSMOSIS BASED METHODS

1.Hypotonic Hemolysis

This approach is predicated on erythrocytes' capacity to experience reversible swelling in a hypotonic solution, as depicted in figure. The extraordinary ability of erythrocytes to undergo reversible shape changes, with or without corresponding volume changes, as well as reversible deformation, is only maintained up to a tonicity of 150 mosm/kg, after which the membrane ruptures, releasing the contents of the cell. A few temporary pores, measuring 200–500, are now produced on the membrane stress, shortly prior to cell lysis.

2.Hypotonic Dilution

The erythrocytes' ability to resist volume is quite low. The quickest and easiest approach is this one. A volume of packed erythrocytes is diluted in this with 2 to 20 volumes of an aqueous medication solution. Erythrocyte membrane ruptures and drug-entrapment holes are produced in hypotonic solution and with volume increases of more than 50–75% of the starting volume. After then, a hypertonic buffer is added to restore the solution's tonicity.

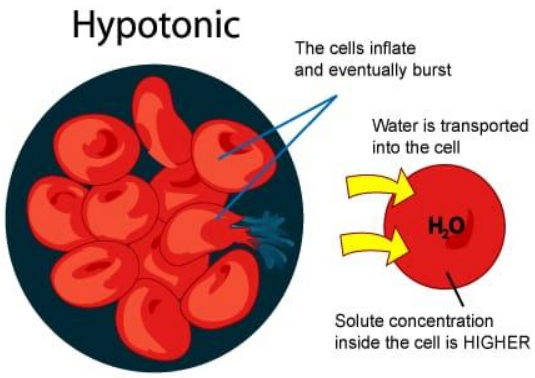


Figure 3: Hypotonic hemolysis

3.Hypotonic Dialysis

In order to load enzymes and lipids, this technique was first described by Klibansky in 1959 and applied by Deloach and Ihlerand Dale in 1977. The idea that semi-permeable dialysis membranes enhance the intracellular: extracellular volume ratio for macromolecules during lysis and resealing underlies a number of techniques. In this procedure, erythrocyte suspension and medication solution are combined to produce the necessary hemocrit. The dialysis tubing is filled with this mixture, and the ends are then secured with thread. There is still an air bubble inside the tube, around 25% of its internal capacity. 100ml of the swelling solution are contained in the container with the tube. For the duration of the desire lysis, the bottle is kept at 4°C. By shaking the tube with the strings, the contents of the dialysis tubing are occasionally intermingled. Next, 100 ml of sealing solution is added to the dialysis tube. The resulting laden erythrocytes are then rinsed with a cold, 4°C phosphate buffer. A good entrapment efficiency is attained with this procedure.

4.Hypotonic Pre-Swelling

This method relies on gradual, controlled swelling in a buffered hypotonic solution. Low g values are used to centrifuge this combination. The supernatant is removed, and 100-120 L parts of an aqueous solution of the medicine to be encapsulated are added to the cell fraction to bring it to the lysis point. Eg: Thyroxin, ibuprofen

B] CHEMICAL PERTURBATION OF THE MEMBRANE

This technique is based on the fact that when erythrocytes are exposed to specific substances, their

membrane permeability increases. In 1973, Deuticke demonstrated that exposure to a polyene antibiotic like amphotericin B enhances the permeability of the erythrocyte membrane. The antineoplastic drug daunomycin was effectively trapped in human and mouse erythrocytes using this technique by Kitao and Hattori in 1980. However, because these techniques result in irreversible damage to the cell membrane, they are not widely employed today.

C] ELECTRO-INSERTION OR ELECTRO ENCAPSULATION

The technique is based on the discovery that a dielectric breakdown caused by an electrical shock causes irreversible alterations in the erythrocyte membrane. The pores can then be re-sealed by 37°C incubation in an isotonic solution. Erythrocytes are suspended in an isotonic buffer during the operation in an electrical discharge chamber. To create a square-wave potential, a capacitor in an external circuit is charged to a specific voltage and then quickly discharged through cell suspension. The ideal discharge time is between 20 and 160 s, and the ideal electric field intensity is between 1 and 10 kW/cm. The duration of the discharge and the electric-field intensity are inversely correlated. From the start of the experiment, the substance to be trapped is added to the suspension medium for the cells.

A membrane of an erythrocyte in compared to osmotic approaches, this method has the advantage of a more uniform dispersion of loaded cells. The complexity of the procedure and the requirement for specialized equipment are the main limitations. This technique has an entrapment efficiency of about 35%, and the lifespan of the resealed cells in circulation is similar to that of healthy cells.

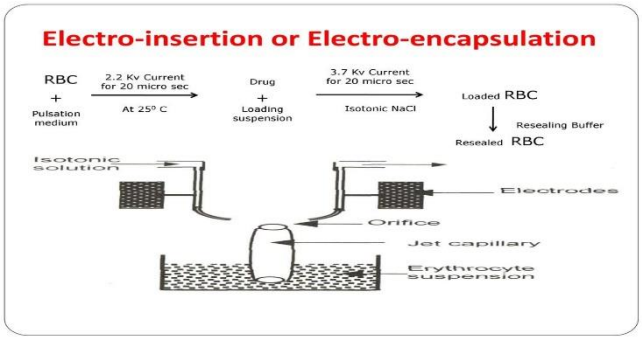


Figure 4: Electro-insertion or Electro encapsulation method

D] ENTRAPMENT BY ENDOCYTOSIS

Schrier reported on this technique in 1975. One volume of washed packed erythrocytes is added to nine volumes of buffer containing 2.5 mM ATP, 2.5 mM MgCl2, and 1 mM CaCl2 for endocytosis, which is then incubated for 2 min at room temperature. By adding 154 mM of NaCl and incubating the pores produced by this procedure for 2 minutes at 37 °C, the pores are resealed. Endocytosis causes the substance to become entrapped. Endocytosis material is kept safe from erythrocytes by being separated from the cytoplasm by the vesicle membrane, and vice versa.

DRUG RELEASE CHARACTERSTICS OF LOADED ERYTHROCYTES

A medication can leave an erythrocyte carrier in one of three major ways: phagocytosis, diffusion across the cell membrane, or usage of a particular transport system. The rate of diffusion is determined by how quickly a specific molecule may pass across a lipid bilayer; it is highest for molecules with high lipid solubility and gradually decreases with increasing polarity or charged group density. Entrapment of strong inhibitors of the relevant transport protein together with the medication may be able to prolong release.

IN VITRO CHARACTERISATION

The biological characteristics of resealed erythrocytes have a significant impact on how well they work in vivo. As a result, in vitro characterization plays a significant role in investigations utilizing these cellular carriers.

A] Physical Characterization

1. Shape and Surface Morphology

The life lifetime of administered erythrocytes is determined by their morphology. Light microscopy of the resealed cells shows no discernible alteration, but in a few instances, spherocytosis (the presence of spherical erythrocytes) is seen. Studies using scanning electron microscopy have revealed that the bulk of the cells keep their biconcave discoid morphologies following the loading technique, while only a small number of stomatocytes develop a type of spherocytosis with an invagination in one place. Microcytes, which are smaller cells, are occasionally seen.

2. Drug content

The effectiveness of the method's trapping depends on the drug content of the cells. Deproteinization of packed, loaded cells (0.5 ml) with 2.0 ml acetonitrile and centrifugation at 2500 rpm for 10 min. are the steps in the procedure. Drug content is examined in the clear supernatant.

3.Deformability

Deformability, or the ability to change shape, is another element that influences a cell's lifespan. This measure assesses how easily erythrocytes flow

through the RES and tiny capillaries. The viscoelasticity of the cell membrane, the viscosity of the cell's contents, and the ratio of the cell's surface to its volume all have a role in how the cells behave rheologically. The deformability is assessed by timing the passage of a predetermined volume of cells through a polycarbonate filter with an average pore size of 45 m or a capillary with a diameter of 4 m. Turbidimetric evaluation of chlorpromazine-induced form alterations is another indirect strategy.

4. Drug Release

The medication release pattern is one of the most crucial factors to consider when evaluating resealed erythrocytes. Because drug release implies the loss of cell membrane integrity, which denotes hemolysis, hemoglobin is likewise invariably liberated. Three general drug release patterns are seen based on the different in vitro release investigations conducted on these cells:

- Hemoglobin releases at a pace that is far lower than that of drugs. In other words, drugs easily disseminate. Lipophilic medications include methotrexate, phenytoin, dexamethasone, primaquin, and vitamin B12 exhibit this trend. The release of these medicines does not require cell lysis.
- The rate of medication release is equivalent to hemoglobin's rate of release. This suggests that cell lysis is necessary for drug release and that drug release cannot occur through simple diffusion. Such a pattern is followed by polar medications such gentamicin, heparin, and enalapril as well as enzymes like asparginase peptides, including urogasterone and lysine-l-phenylalanine.
- Between the aforementioned two extremes, the rate of drug release is found in substances like propranolol, isoniazid, metronidazole, and recombinant human erythropoietin.

B] Cellular characterization

1.Osmotic Fragility

Resealed erythrocytes' osmotic fragility is a sign of potential alterations in the integrity of their cell membranes and a measure of how well they can withstand the osmotic pressure of the suspension medium. To conduct the test, cells are suspended in media with various salt chloride concentrations, and the amount of hemoglobin released is measured. Due to increased intracellular osmotic pressure, resealed cells typically have higher osmotic fragility than normal cells.

2.Turbulent Fragility

Another trait, turbulence fragility, is dependent on modifications to the integrity of cellular membrane and represents the resistance of loaded cells to hemolysis brought on by turbulent flow within circulation. It is determined by either vigorously shaking the cell suspension or passing the suspension through needles with smaller internal diameters (for example, 30 gauges). Hemoglobin and medication discharged during the surgery are identified in both situations. It is discovered that resealed cells have a higher turbulent fragility.

3. Percent cell recovery

It can be determined by counting the number of intact cells per cubic mm of packed erythrocyte before and after loading the drug.

C] Biological characterization

Sterility tests, pyrogen tests utilizing the rabbit method, LAL tests, and toxicity tests on animals can all be used to accomplish this.

IN VIVO CHARACTERISATION:

The amount of time that resealed erythrocytes remain in the bloodstream after being reinjected determines primarily how effective they are. Cells can be marked with fluorescent markers like fluorescin isothiocyanate or 51Cr, or they can be trapped in 14C sucrose or gentamicin to measure in vivo survival time. Resealed erythrocytes' circulation survival kinetics exhibit typical bimodal behavior, with a rapid loss of cells within the first 24 hours after injection, followed by a steady decline phase with a half-life on the scale of days or weeks.

Route of administration

The experimental animals have typically received resealed erythrocyte intravenously through the cardinal vein throughout experiments. DeLoach used a subcutaneous method to release entrapped drugs gradually. He examined how interleukin-2 behaved after being injected subcutaneously into mice. Propranolol nasal administration based on erythrocytes was recently proposed by Talwar (1993).

In vitro storage

Resealed erythrocytes' in vitro preservation has a larger role in how well they work as a drug delivery method. Hank's balanced salt solution and acid-citrate-dextrose at 4°C are the most typical storage media. For at least two weeks at this temperature, cells retain their physiologic and carrier features. The circulatory survival time of cells after reinjection is improved by the addition of purine nucleosides or calcium-chelating compounds. It has been observed that lyophilization or sintered glass filtration, followed by exposure to membrane stabilizing chemicals including dimethyl sulfoxide, dimethyl,3,3-dithio bispropionamide, gluteraldehyde, and toluene-2-4-

diisocyanate, increases the stability of resealed erythrocytes when stored. Additionally, cells suspended in oxygenated HBBS with 1% soft gelatin can be used to maintain the material. After the gel has been liquefied by putting the tube in a water bath set at 37°C and centrifuging it, the cells have recovered well. Cryopreservation of RBCS in liquid nitrogen has been used as an additional storage technique.

APPLICATIONS OF RESEALED ERYTHROCYTES

There are numerous potential uses for resealed erythrocytes in both human and veterinary medicine. Such cells could be utilized as circulating carriers to spread a medicine over a long length of time in the bloodstream or in organs with a particular target, such as the lymph nodes, spleen, and liver. The majority of the erythrocyte-based medication delivery trials are still in the preclinical stage. There have been several clinical investigations with positive outcomes.

In-vitro applications

Because cells that have undergone in vitro phagocytosis have been utilized to help phagolysosomes absorb enzymes. The use of cytochemical approach allowed for the visualization of the enzyme content within carrier RBC. RBCs are most frequently used for microinjections in vitro. Through the fusion process, a protein or nucleic acid was introduced into eukaryotic cells. Similar to this, when antibody molecules are supplied via the erythrocytic carrier system, the cytoplasm is instantly invaded by them.

In–vivo applications

- ✓ RES System-specific targeting of bioactive substances
- ✓ targeting locations other than organs with high RES
- ✓ A Circulating Bioreactor: Erythrocytes
- ✓ Erythrocytes as Drug Transporters
- ✓ Erythrocytes as Enzyme Carriers
- ✓ targeting to the Erythrocytes as Protein and Macromolecule Carriers.

Drug targeting to the RES organs

1] slow Drug Release

The continuous delivery of antineoplastics, antiparasitics, veterinary antiamoebics, vitamins, hormones, antibiotics, and cardiovascular medicines has been accomplished by using erythrocytes as circulating depots.

The various mechanisms proposed for drug release include-

- Passive diffusion.
- Specialized membrane associated carrier transport.
- Phagocytosis of RES macrophages that have been resealed, drug buildup in the inside of the macrophage, and delayed release of the drug.
- Accumulation of erythrocytes in lymph nodes upon subcutaneous administration followed by hemolysis to release the drug.

2] Targeting to the liver

By injecting these enzymes, many metabolic diseases caused by insufficient or absent enzymes can be addressed. However, exogenous enzyme therapy has drawbacks such as a shorter enzyme circulation half-life, allergic responses, and toxic symptoms. The enzymes can be given as resealed erythrocytes to successfully overcome these issues. Glucosidase, glucoronidase, and galactosidase are the enzymes that are utilized.

3] Treatment of hepatic tumors

Hepatic tumors are one of the most prevalent types of cancer. Antineoplastic drugs such as methotrexate, bleomycin, asparginase, and Adriamycin have been successfully delivered by erythrocytes. Agents such as daunorubicin diffuse rapidly from the cells upon loading and hence pose a problem. This problem can be overcome by covalently linking daunorubicin to the erythrocytic membrane using glutaraldehyde orcisaconitic acid as a spacer. The resealed erythrocytes loaded with carboplatin show localization in liver.

4] Treatment of parasitic diseases

The ability of resealed erythrocytes to selectively accumulate within RES organs make them useful tool during the delivery of antiparasitic agents. Parasitic diseases that involve harboring parasites in the RES organs can be successfully controlled by this method. Results were favorable in studies involving animal models for erythrocytes loaded with antimalarial, antileishmanial and anti-amoebic Drugs.

5] Removal of RES iron overload

Erythrocytes that have been loaded with desferrioxamine have been used to treat excess iron that has built up as a result of numerous transfusions to thalassemic patients. It is particularly advantageous to target this medication to the RES since these organs accumulate iron because elderly erythrocytes are destroyed there.

6] Removal of toxic agents

Cannon observed that mouse carrier erythrocytes containing bovinerhodanase and sodium thiosulfate prevented cyanide poisoning. It has also been observed that resealed erythrocytes harboring recombinant phosphodiestrase can inhibit organophosphorus poisoning.

7] Delivery of antiviral agents

While nucleotides do not traverse the membrane as quickly as nucleosides do, they have longer release profiles as a result. These moieties must be changed into purine or pyrimidine bases in order for nucleotides to be released. Deoxycytidine derivatives, recombinant HSV-1 glycoprotein B, azidothymidine derivatives, azathioprene, acyclovir, and fludarabine phosphate have all been administered using resealed erythrocytes.

8] Enzyme replacement therapy

Enzymes are frequently employed in clinical practice as medications, replacement therapies to treat disorders caused by their lack (such as Gaucher's disease and galactosuria), and to break down hazardous substances after poisoning (such as cyanide and organ phosphorus). It has been noted that there are issues with injecting enzymes directly into the body. Using erythrocytes that have been loaded with enzymes is one way to solve these issues. Following hemolysis, these cells either operate as "circulating bioreactors" in which substrates enter the cell, interact with enzymes, and produce products, or they store enzymes in RES for later catalysis. The treatment of Gaucher's disease with β- glucoserebrosidase is the first report of successful clinical trials using resealed erythrocytes laden with enzymes for replacement therapy. Lysosomal β -glucoserebrosidase deficiency in RES cells, which causes a buildup of β-glucoserebrosidase in RES macrophages, is the disease's defining feature.

9] Improvement in the oxygen delivering to tissues

The protein known as hemoglobin is what gives erythrocytes their ability to carry oxygen. In the lungs, 95% of hemoglobin is saturated with oxygen under normal circumstances, but only 25% of oxygenated hemoglobin deoxygenates in peripheral circulation under physiological settings. As a result, venous blood reticulates the majority of the oxygen linked to hemoglobin to the lungs. It has been suggested that this bound fraction be used to treat oxygen deprivation. Hemoglobin naturally produces 2, 3-Diphosphoglycerate (2, 3-DPG). variations in the intracellular concentration of 2,3-DPG cause reversible variations in hemoglobin's binding affinity for oxygen. As 2; 3-DPG has a significantly higher affinity for oxygen than hemoglobin does, this compensates for variations in the oxygen pressure outside the body.

10] Microinjection of macromolecules

Erythrocytes are injected into the host cells using a microinjection technique. Host eukaryotic cells are in vitro cultured during the microinjection procedure. The cells are coated with a fusogenic agent and then suspended in an isotonic medium with erythrocytes that have been loaded with the desired substance. Fusogenic agents have been created using the Sendai virus (also known as the Hemagglutinating Virus of Japan, HVJ), its glycoproteins, or polyethylene glycol. Co-suspended erythrocytes and eukaryotic cells are fused by the fusogen. Thus, the compound of interest and the contents of the resealed erythrocytes are transported to the host cell. DNA fragments, arginase, proteins, nucleic acids, ferritin, latex particles, bovine and human serum albumin, and the enzyme thymidine kinase have all been microinjected into different eukaryotic cells using this technique.

11] Applications of carrier Erythrocytes in delivery of biopharmaceuticals

According to the scientist, investigations and laboratory experiences on successful erythrocyte loading and characterization of the various types of biopharmaceuticals have been highlighted as they pertain to the possible applications of erythrocytes in medication delivery.

NOVEL APPROACHES ERYTHROSOMES

Erythrosomes are specially designed vesicular systems in which a lipid bilayer is coated on top of chemically cross-linked human erythrocyte cytoskeletons. A modification method typically used for reverse phase evaporation can be used to accomplish this. In especially for macromolecular pharmaceuticals, erythrosomes are suggested as a viable encapsulating mechanism for drug delivery. By covering cross-linked erythrocyte cytoskeletons with phosphatidyl choline, large (3, tm diameter) mechanically stable proteoliposomes (erythrosomes) were successfully produced.

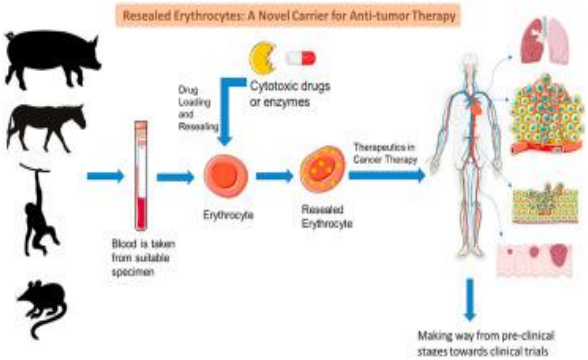


Figure 5: Resealed erythrocytes: A novel carrier for Anti-tumor Therapy

Application

- On experimental animal models, pentamidine, primaquine phosphate, and metronidazole have been used to treat malaria and leshmaniasis.

- It can act as the perfect vehicle for anti-cancer medications like bleomycin.
- In RBCs, steroids and vitamins have been encapsulated.
- It acts as a cellular sustained delivery system for recombinant erythropoietin when administered in vivo.
- Recombinant interlukin-2-coated RBCs are said to offer sustained release, enabling low and harmless concentrations of rlL-2 in the bloodstream.

Nanoerythrosomes (nEs)

The extrusion of RBC ghost creates nanoerythrosomes, which have vesicles with an average diameter of 100 nm. Small vesicles the size of liposomes were produced by the technique. Using specific cross-linking chemicals like gluteraldehyde, which has better activity than free drug and offers higher stability, the pharmaceuticals can be attached to the Nanoerythrosomes. The Nanoerythrosomes are evaluated for a number of characteristics, including surface shape, drug conjugation percentage, centrifugal stress, in vitro release, etc. Thus, nEs are extremely adaptable drug delivery systems (DDS) or bioactive drug carriers. These nEs are persistent and preserve daunorubicin's cytotoxic and anti-cancer effects on mice leukemia p338D cells. The compositions of nanoerythrosomes also facilitate the development of bioassays. Heparin and glucocortisone were both encapsulated in erythrocytes in the instance of Gaucher disease to prevent thrombosis.

Preparation of (NES) and drug loading

1.Extrusion

Under nitrogen pressure, the erythrocyte ghost suspension (50 percent haematocrit) is extruded through a 25 mm polycarbonate membrane filter with a 0.4 m pore size. Uranyl acetate is used to dye the ghosts, which are then examined under a microscope. The final preparation is kept at extrusion apparatus at 37°C. Extrusions are carried out in a thermostatically controlled extrusion equipment at a temperature of 37°C.

2. Sonication

Using a dismembrator, erythrocyte ghosts are transformed into tiny vesicles.

3. Electrical breakdown method

It is utilized to transform ghosts into tiny vesicles when an electric potential is present.

Advantages

1. Non immunogenic in action and able to target diseased organ/tissue.
2. Extend the drug's duration of systemic activity while staying in the body for a longer period of time.
3. Prevent protein and enzyme inactivation, premature breakdown, and excretion.
4. Concentrate the medications both in RES and in non-RES organs and sites.
5. There is no potential for a triggered immune response.
6. The drug and the erythrocyte membrane protein are chemically linked.
7. Less prone to fusion and aggregation.
8. The membrane's flexibility enables them to evade RES for longer periods of time.

Applications

Resealed erythrocytes are fairly simple to prepare. Today, a number of methods have been discovered that make it simple to include the medication into erythrocytes. Both in vitro and in vivo applications are available for it. Traditional medication delivery methods had a number of drawbacks, including significant toxicity and a high minimum effective dose. The most crucial of the numerous carriers are those that, at the very least, match the body's inherent components and prevent an immunological reaction when administered intravenously. Patient non-compliance with many pharmacological regimens is a drawback caused by increased dose frequency.

DISCUSSION:

Resealed erythrocytes are fairly simple to prepare. Today, a number of methods have been discovered that make it simple to include the medication into erythrocytes. Both in vitro and in vivo applications are available for it. Traditional medication delivery methods had a number of drawbacks, including significant toxicity and a high minimum effective dose. The most crucial of the numerous carriers are those that, at the very least, match the body’s inherent components and prevent an immunological reaction when administrated intravenously. Patient an immunological reaction when administrated intravenously. Patient non-compliance is a drawback of many pharmacological regimens since they require frequent doses. Resealed erythrocytes are and a high minimum effective dose.

CONCLUSION:

For a secure and reliable administration of diverse medications for passive and active targeting, the use of resealed erythrocytes appears promising. To become a standard drug delivery method, the idea needs to be improved. The same idea can be applied to how biopharmaceuticals, proteins, and steroids are delivered. It was discovered that using resealed

erythrocytes as a novel drug delivery method was very advantageous for targeting drug delivery systems based on sustain release insulin, antiviral agent, oxygen deficiency therapy, etc.

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